REMARKS

Claims 1-10, 13-22 are pending. Claims 1-9 and 17-22 were previously withdrawn by the Examiner. Claim 10 has been amended to clarify the claimed process. In particular, claim 10 as amended clarifies that the process is for the quantitative preparation of a conjugate of one or two molecular entities imparting a diagnostic activity and a Fab fragment (e.g. a mono- or di-conjugate) and it recites the concentrations of TCEP, Fab and diagnostic molecular entity necessary to achieve this selective reduction.[1] Support is found throughout the specification, particularly paragraphs 0037-0039, in Examples 5 and 6 and in original claims 2 and 3. Support for the concentration ranges is found at paragraphs 0044-0045 and 0053. Claims 13-14 and 16 have been amended to be consistent with claim 10 and to clarify the temperature specified. These amendments are supported throughout the specification and particularly at paragraph 0056 and in the Examples. No new matter has been added.

Withdrawn Rejections

Applicants are grateful for the withdrawal of the rejection of claims 10 and 13-16 under 35 U.S.C. 112, first paragraph and for the withdrawal of the rejection of claims 10 and 13-16 under 103(a) for alleged obviousness over Liu et al. (Journal of Labeled Compounds and Radiopharmaceuticals 1998: XLI:37-45) in view of Getz et al. (Analytical Biochemistry 1999;273:73-80 – "Getz") and Maurer et al. (WO 02/056907 A2 – "Maurer") as evidenced by Cruse and Lewis (Cruse, Julius and Lewis, Robert. Illustrated Dictionary of Immunology Boca Raton, FL 1995 – "Cruse and Lewis").

Rejections Under 35 U.S.C. § 103

Claims 10 and 13-16 were rejected for alleged obviousness over Gray et al. (US Patent 5,380,513 – "Gray") in view of Getz and Maurer as evidenced by Cruse and Lewis. Applicants respectfully traverse.

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[1] Applicants have deleted the language regarding the stoichiometric molar ratio as this language was introducing unnecessary confusion: it was intended to refer to the stoichiometric molar ratio between the Fab and the diagnostic molecular entity in the final mono- or di-conjugate; however the Examiner was applying it to the concentration of the component species in the reaction mixture. Applicants submit that the claims as amended clarify the invention.

"The key to supporting any rejection under 35 U.S.C. 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. The Supreme Court in KSR International Co. v. Teleflex Inc., 82 USPQ2d 1385, 1396 (2007) noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit." MPEP Section 2143.

"The rationale to support a conclusion that the claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art." 83 UDPQ2d at 1395 and MPEP Section 2143. Further, a prima facie case of obviousness based on structural similarity is rebuttable by proof that the claimed compounds possess unexpectedly advantageous or superior properties. (MPEP 2144.09).

Applicants respectfully assert that the combination of the cited references does not teach or suggest all of the claim limitations and that the claimed process has unexpectedly superior properties. Therefore, applicants respectfully traverse the § 103 rejection.

The pending claims require a process for the quantitative preparation of a conjugate of one or two molecular entities imparting a diagnostic activity and a Fab fragment (e.g. a mono- or di-conjugate) comprising:

a) reducing the inter-chain disulfide bond of a Fab fragment at a concentration of 1.5 to 10 μM using TCEP in a concentration of 0.1 – 10 mM;

b) functionalizing one or both of the sulfhydryl groups from step a) with said molecular entities in a concentration of 0.1 to 100 mM wherein said molecular entities have at least one free sulfhydryl-reactive group and impart diagnostic utility; and

c) quantitatively obtaining a mono- or di-conjugate compound, said diconjugate deriving from either symmetric or asymmetric functionalization of the sulfhydryl groups.

Thus, this process selectively reduces the inter-chain disulfide bond, leaving the intra-chain disulfide bonds intact. The functionalization of this Fab results in a homogeneous product that, unlike the prior art processes, does not include substantial amounts of unreacted Fab and/or diagnostic entity or species in which multiple diagnostic molecular entities are

conjugated to the Fab. Consequently this process obviated the need for the extensive purification required in the prior art processes. Moreover, functionalization at only the reduced inter-chain disulfide, located in the C-terminal region, is unlikely to affect the antigen binding recognition site.

None of the cited references whether considered alone of in combination teach or suggest all elements of the claimed process. As the Examiner admits (OA, p. 3), Gray fails to disclose use of TCEP as the reducing agent as required by the claims and it fails to disclose the claimed concentration of Fab (1.5 to 10 μM), molecular entity (0.1 to 100 μM) and TCEP (0.1 to 10 mM). Indeed Gray fails to disclose any specific concentrations or reaction conditions and fails to mention or suggest the need for a selective reduction of inter-chain disulfide bonds as required in the claimed process. Thus there is no teaching or suggestion in Gray of a process for the quantitative preparation of a mono- or di-conjugate as claimed.

Getz fails to teach or suggest the conjugate of a Fab fragment and a recited molecular entity imparting diagnostic utility required by the claims. Moreover, Getz is directed to reduction of myosin and fails to teach or suggest use of TCEP for reduction of inter-chain disulfide bonds in antibodies, let alone the claimed selective reduction of only inter-chain disulfide bonds in Fabs as required by the method of the claims. Getz also fails to teach or suggest the claimed concentrations of Fab, molecular entity and TCEP which are required to selectively reduce only the inter-chain (and not intra-chain) disulfide bonds. Getz also fails to teach or suggest the conditions of claims 13-16.

Maurer fails to remedy these deficiencies. Maurer does not disclose a process for quantitatively preparing a mono- or di- conjugate between an immunoglobulin Fab fragment and a diagnostic moiety as defined in the claims. Maurer is directed to producing conjugates for vaccines, a therapeutic utility, and neither teaches nor suggests the recited diagnostic conjugates.

Moreover, Maurer fails to teach or suggest that TCEP can be used to selectively reduce only the inter-chain disulfide bond of a Fab fragment. Similarly, Maurer fails to teach or suggest the concentration of Fab, diagnostic molecular entity and TCEP required for this selective reduction. Consequently, the Maurer method yields a heterogeneous mixture of species, including the starting materials (unconjugated Fab and Qβ capsid protein) as well as high molecular weight species (presumably conjugates of the Fab with multiple Qβ capsid proteins) and, as only a minor amount, the desired conjugate. Indeed, Example 16 and Figure 21

of Maurer establish that each of the "successful" couplings between the Fab fragment and the protein using TCEP (see lanes 5-8 of Figure 21) results in a complex mixture of species and only a small fraction of these are the desired conjugate of Fab and Qβ capsid protein identified by the arrow. In each of these lanes, higher molecular weight species are present (presumably conjugates with multiple Qβ capsid proteins) as well as huge amounts of unreacted Fab (the 25 kD band) and unreacted Qβ capsid protein (the band below 16kD). Consequently the Maurer process, even when TCEP is used, requires extensive, expensive and impractical separation methods to isolate the derivative of interest from the complex mixture containing it. There is no teaching or suggestion in Maurer or any of the other references that by modifying the concentrations or other reaction conditions a selective reduction of just inter-chain disulfide binds bonds could be obtained. Thus, contrary to the Examiner's assertion (OA, p. 6), based on the combination of Gray, Getz and Maurer one skilled in the art would have no motivation to substitute TCEP in the process of Gray and even if such substitution was made there would be no expectation of success as none of the cited references teaches or suggests the claimed conditions, which enable use of TCEP to reduce only the inter-chain disulfide bonds..

In contrast, in the present invention, the claimed method unexpectedly allows quantitative preparation of a mono- or di-conjugate of a Fab and a diagnostic molecular entity by 1) using TCEP and the conjugate components in the claimed concentrations to selectively reduce only the inter-chain disulfide bond; and 2) functionalizing one or both of the sulfhydryls from the reduced inter-chain disulfide bond with one or two diagnostic molecular entities. The result of this process is a reaction mixture that unexpectedly contains only the desired mono- or di-conjugate and no unreacted components; thus obviating the need for the extensive purification steps required with Maurer and indeed all cited prior art processes. These unexpected results are shown in, for example, Figure 1 of the instant application, which shows cation-exchange HPLC analyses of: Panel A, the compete reaction mixture resulting from the process of the invention (and specifically of Example 1), Panel B, the initial unreacted fab solution and Panel C, the solution containing the unreacted Fab and unreacted β-maleimidoproprionic acid. By comparing the panels of Figure 1, it is clear that the final reaction mixture in Panel A includes only a single main peak, indicating a homogeneous product and, importantly, it does not include any unreacted Fab (main peak at about 10 in Panel B). This result was unexpected in view of the complex mixture obtained in the Maurer process even when TCEP was used

The tertiary reference Cruse and Lewis fails to remedy the deficiencies of Maurer, Liu and Getz. Indeed, it was cited by the Examiner simply for the molecular weight of the Fab of Maurer and fails to teach or suggest the claimed methods or the recited conjugates.

In sum, whether taken alone or together, the cited references fail to teach or suggest the claimed methods which quantitatively provide mono- or di-conjugates of a Fab and a diagnostic molecular entity through the selective and quantitative reduction of only the inter-chain disulfide bond of a Fab fragment using TCEP at concentrations of 0.1 to 10 mM, Fab concentrations of 1.5 to 10 μM and diagnostic molecular entity concentrations of 0.1 to 100 μM.

In view of the present amendments and foregoing remarks, reconsideration of the rejections and advancement of the case to issue are respectfully requested.

No fee is believed to be necessary in connection with the filing of this Amendment and Response to Restriction Requirement. However, if any additional fee is necessary, applicant hereby authorizes such fee to be charged to Deposit Account No. 50-2168.

Respectfully submitted,

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